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THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 268, No. 23, Issue of August 15, pp. 17623-17627, 1993
Printed in U.S.A.

Isolation and Characterization of a cDNA Encoding Rat Liver Cytosolic Epoxide Hydrolase and Its Functional Expression in *Escherichia coli**

(Received for publication, December 3, 1992)

Michael Knehr‡, Helmut Thomas§, Michael Arand¶, Thomas Gebel‖, Hans-Dieter Zeller, and Franz Oesch**

From the Institute of Toxicology, University of Mainz, W-6500 Mainz, Germany

A cDNA of 1992 base pairs encoding the complete rat liver cytosolic epoxide hydrolase has been isolated using a polymerase chain reaction-derived DNA fragment (Arand, M., Knehr, M., Thomas, H., Zeller, H. D., and Oesch, F. (1991) *FEBS Lett.* 294, 19–22) known to represent the 3′-end of the cytosolic epoxide hydrolase mRNA. Sequence analysis revealed an open reading frame of 1662 nucleotides corresponding to 554 amino acids (M_r = 62,268). The DNA sequence obtained did not display significant homology to the sequences of microsomal epoxide hydrolase or leukotriene A_4 hydrolase or to any other DNA included in the EMBL Data Bank (release 32). On Northern blotting of rat liver RNA, a single mRNA species was detected that was strongly induced on treatment of the animal with fenofibrate, a potent peroxisome proliferator. The most significant structure of the deduced protein is a modified peroxisomal targeting signal (Ser-Lys-Ile) at the carboxyl terminus that is regarded to be responsible for the unusual dual localization of the cytosolic epoxide hydrolase in peroxisomes as well as in the cytosol. In addition, a leucine zipper-like motif was identified at the amino terminus. Its possible implication for the observed dimeric structure of cytosolic epoxide hydrolase is discussed. The isolated cDNA was expressed in bacteria to yield a catalytically active enzyme. Specific activity of the crude lysate obtained exceeded that of rat liver cytosols from maximally induced animals by a factor of 8.

Mammalian epoxide hydrolases (EC 3.3.2.3) comprise a heterogeneous group of enzymes capable of metabolizing endogenous and exogenous epoxides to vicinal *trans*-diols by the addition of water (1–6). The formation of epoxides is often the first step in the metabolic cascade by which lipophilic substances are converted to more water-soluble, readily excretable compounds in higher organisms. These epoxides include highly electrophilic species with a pronounced potential to react with biological macromolecules, *i.e.* proteins and nucleic acids. The resulting cytotoxic and mutagenic potential calls for an efficient mechanism for the rapid and efficient inactivation of the toxic agents. Epoxide hydrolases, together with glutathione *S*-transferases, represent the detoxifying system that protects the organism from the destructive effects of those reactive intermediates (7–9).

Among the different epoxide hydrolases known to date, cytosolic epoxide hydrolase (cEH)[1] has a number of unique characteristics. The rat liver enzyme is a soluble homodimeric protein with an estimated native M_r of 120,000 and an apparent subunit M_r of 61,000 (10). Its substrate specificity includes epoxides derived from unsaturated fatty acids (11–13), polycyclic aromatic compounds (14, 15), and *trans*-substituted styrene derivatives, such as *trans*-stilbene oxide (16), which is widely used as a marker substrate. In contrast to many other xenobiotic-metabolizing enzymes, cEH is not inducible by the prototype inducers phenobarbital and 3-methylcholanthrene or the microsomal epoxide hydrolase inducer *trans*-stilbene oxide, but is induced concomitantly with the peroxisomal β-oxidation by peroxisome proliferators such as clofibrate, fenofibrate, and tiadenol (17, 18). In rat liver, where the constitutive expression of cEH is especially low as compared to the situation in other mammals, an increase in cEH activity by a factor of up to 13 has been reported (18).

The subcellular localization of cEH has been the subject of numerous investigations. The enzyme was first discovered in liver cytosolic preparations (2), but was later detected in the mitochondrial fraction as well (19). Further experiments elucidated that the majority, if not all, of the "mitochondrial cEH" was associated with peroxisomes (20–23). No significant difference between the individual cEH forms with respect to their physicochemical, immunological, and biochemical characteristics could be demonstrated by a variety of investigators (24–26). Thus, instability of peroxisomes upon liver fractionation with the consequence of cEH leakage or the existence of two very similar isozymes were proposed as possible explanations for the peculiar subcellular distribution of cEH. Recently, we demonstrated the presence of a modified peroxisomal targeting signal at the carboxyl terminus of cEH (27) that is impaired in its targeting efficiency, as shown by others for a different system (28). This observation favors the assumption of a nonquantitative import of cEH into peroxisomes as the reason for the observed bicompartmental distribution.

Up to now, only limited information was available on the cEH structure (amino acid composition (29) and prediction of the

* This work was supported in part by German Research Council Grant SFB 302. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X65083.

‡ Recipient of a postgraduate stipend of the Astrid-Haugstrup-Sörensen-Gedächtnisstiftung awarded by the Stifterverband für die Deutsche Wissenschaft.

§ Present address: Ciba-Geigy AG, R-1066.4.32, CH-4002 Basel, Switzerland.

¶ To whom correspondence should be addressed: Inst. of Toxicology, University of Mainz, Obere Zahlbacherstra. 67, W-6500 Mainz, Germany.

‖ Recipient of a postgraduate stipend from the Claussen-Foundation.

** Present address: Boehringer Mannheim GmbH, Dept. BN-Q, Nonnenwald 2, D-8122 Penzberg, Germany.

[1] The abbreviations used are: cEH, cytosolic epoxide hydrolase; bp, base pair(s).

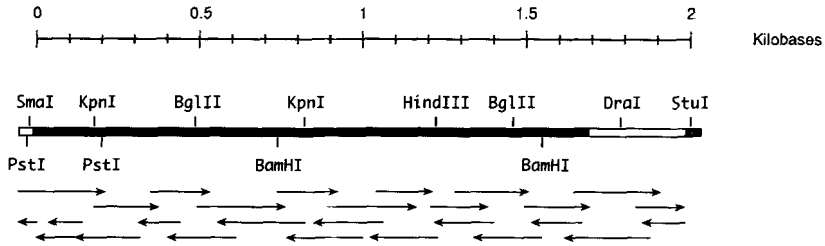

FIG. 1. **Restriction map and sequencing strategy for rat liver cytosolic epoxide hydrolase.** The scale at the top designates the nucleotide position in kilobases from the start codon of the sequence. The *solid box* indicates the open reading frame, and the *open boxes* represent the untranslated regions of the sequence. The *stippled box* at the 3'-terminus indicates the part of the sequence that is not contained within clone pUCcEH1, but has been determined from a polymerase chain reaction-derived cDNA as described earlier (27). The *arrows* represent the direction and the extent of selected separate sequence determinations, sufficient for the continuous reading of both strands. Additional determinations have not been shown in the interest of clarity.

percentage of α-helical regions (30)). Initial attempts to isolate specific cDNAs from a rat liver expression library using a polyclonal anti-cEH antiserum (31) finally failed.[2] Recently, we used limited sequence information obtained from cEH peptide analysis to set up polymerase chain reaction experiments that resulted in the isolation of a specifically amplified DNA fragment that was shown to represent the 3'-end of the cEH mRNA (27). We now describe the isolation and analysis of a cDNA carrying the complete coding sequence for rat liver cEH and its functional expression in *Escherichia coli*.

EXPERIMENTAL PROCEDURES

Construction and Screening of Rat Liver cDNA Library—Two 6-week-old male Sprague-Dawley rats were fed a tiadenol-containing diet (0.1%, w/w) for 7 days to induce cytosolic epoxide hydrolase as described earlier (18). Thereafter, the animals were killed by cervical dislocation to isolate RNA from their livers as described by Chirgwin *et al.* (32). Messenger RNA was selected by standard oligo(dT)-cellulose chromatography (33) and was taken to construct cDNA with the aid of a commercial kit (You Prime cDNA synthesis kit, Pharmacia LKB Biotechnology Inc.) using a mixture of oligo(dT)$_{18}$ and a cEH 3'-end-specific oligonucleotide (CTTCCTTCCCAACG) deduced from our previous work (27) for the initiation of the first-strand synthesis. The cDNA was inserted into the *Eco*RI site of pUC19 using *Not*I/*Eco*RI adaptors to yield a library of 3.6×10^7 independent clones with a 35% frequency of recombinants after transformation into *E. coli* C600. The 420-bp *Dra*I fragment of the polymerase chain reaction-derived cEH cDNA described earlier (27) was labeled with digoxigenin-dUTP and used to screen the cDNA library under stringent conditions according to the recommendations of the manufacturer (Boehringer Mannheim). Colonies giving specific hybridization signals with the probe were isolated, and the size of their cDNA insert was determined by restriction analysis with *Not*I, a rare cutter with a recognition site in the cloning adaptor.

Sequence Analysis—Dideoxy sequencing (34) of cDNA in pUC19 was carried out using a commercial kit (T7 sequencing kit, Pharmacia). First, cDNAs were fragmented using appropriate restriction enzymes to yield inserts of sizes below 800 bp.[3] Determination of their sequences led to the identification of additional restriction sites that were used for further subcloning to allow for the overlapping sequencing of both strands. Final gaps among the sequences obtained were closed by the construction of respective oligonucleotides that were used as primers for additional sequencing reactions. Sequence assembly and analysis were performed using the GeneWorks 2.1 program (Intelligenetics) on a Macintosh SE/30 microcomputer.

Northern Blot Analysis—Male Sprague-Dawley rats were fed a fenofibrate diet (0.25%) for 0–12 days, and RNA was isolated from their livers according to Chomczynski and Sacchi (35). Northern blotting of the resulting RNA samples was carried out after denaturing electrophoresis on 2.2 M formaldehyde-agarose gels essentially as described (36) using the digoxigenin-labeled insert of clone pUCcEH1 at a concentration of 10 ng of probe/ml of hybridization solution. Stringency washes were performed two times, for 15 min each, at 65 °C in 0.1% SDS, 0.1 × SSC (SSC = 0.15 M NaCl and 0.015 M sodium citrate).

Expression of pUCcEH1 cDNA—The 1969-bp *Sma*I/*Eco*RI cDNA fragment of pUCcEH1 was inserted into the *Puu*II/*Eco*RI site of the bacterial expression vector pRSET B (Invitrogen). After transformation of *E. coli* JM109, expression was initiated in liquid culture at exponential growth by supplementation with isopropyl-1-thio-β-D-galactopyranoside (1 mM final concentration) and subsequent infection with M13/T7, a recombinant M13 phage carrying an isopropyl-1-thio-β-D-galactopyranoside-inducible T7 RNA polymerase gene (Invitrogen), at a multiplicity of infection of 5. The level of cEH expression in the bacterial culture was monitored over an 18-h period by quantification of the enzymatic hydrolysis of *trans*-stilbene oxide essentially as described earlier (37) using the crude bacterial suspension as the source of enzyme without any further treatment prior to analysis. Protein concentrations were determined according to Bradford (38).

RESULTS

Isolation and Analysis of pUCcEH1—The colony screening of the self-constructed rat liver cDNA library resulted in the isolation of 14 specifically hybridizing clones carrying cDNA inserts ranging in size from 0.5 to 2.0 kilobases. Sequencing of the largest isolate, designated pUCcEH1, revealed a 1992-bp cDNA containing an open reading frame of 1662 bp. The encoded polypeptide of 554 amino acids has a calculated M_r of 62,268 and a calculated pI of 5.8. All cEH peptide sequences determined in a previous study (27) could be identified in the encoded sequence. The coding frame was preceded by 41 bp, with the ATG codon situated in a sequence context favorable for translation initiation in eucaryotes (39). By comparison to the polymerase chain reaction-derived cDNA isolated earlier (27), the 3'-end of the pUCcEH1 cDNA was located 11 bases upstream of the polyadenylation signal of the cEH mRNA. The restriction map and nucleotide and deduced amino acid sequences of the cDNA for rat liver cEH are presented in Figs. 1 and 2, respectively.

Sequence Comparison with Other Epoxide Hydrolases—Forced alignments of the cEH nucleic acid and deduced amino acid sequences with those of microsomal epoxide hydrolase from rat (40) and leukotriene A_4 hydrolase from mouse (41) revealed no detectable phylogenetic relationship among the three different epoxide hydrolases. The degree of similarity did not exceed results obtained with randomly chosen nucleic acid or protein sequences of no relationship. Furthermore, no significant homology to other nucleic acid sequences in the EMBL Data Bank (release 32) could be identified on similarity search.

Structural Features—At the carboxyl terminus of the cEH, the impaired peroxisomal targeting signal Ser-Lys-Ile was identified (see Fig. 2), confirming our previous findings (27). On search for PROSITE motifs, a leucine zipper motif (42) was identified near the amino terminus of the protein (amino acids 16–37) (see Fig. 2). Secondary structure prediction using the algorithms of Garnier *et al.* (43) identified ~40% of the total

[2] M. Knehr, unpublished data.

[3] At that time, B. Hammock and D. Grant (Institute of Entomology, University of California, Davies, CA) made the sequence of an almost complete mouse liver cEH cDNA available to us, which substantially facilitated the choice of convenient restriction sites due to the extensive homology among the rat and mouse enzymes.

```
                -41  CTTCTTGTCTTTGTCAGCTTGGCGCTGCAGCCCGGGCCATC ATG GCG CTG CGT GTG GCC GCG TTC GAC CTT        30
                                                             Met Ala Leu Arg Val Ala Ala Phe Asp Leu        10

GAC GGA GTG CTG GCC CTC CCC TCT ATA GCC GGG GTT CTG CGC CAC ACC GAG GAG GCC CTG GCG CTG CCC AGA GAC        105
Asp Gly Val Leu Ala Leu Pro Ser Ile Ala Gly Val Leu Arg His Thr Glu Glu Ala Leu Ala Leu Pro Arg Asp         35

TTC CTA CTT GGC GCT TTC CAG ATG AAA TTC CCA GAG GGA CCC ACT GAG CAA CTC ATG AAA GGA AAG ATC ACA TTT        180
Phe Leu Leu Gly Ala Phe Gln Met Lys Phe Pro Glu Gly Pro Thr Glu Gln Leu Met Lys Gly Lys Ile Thr Phe         60

TCC CAG TGG GTA CCA CTC ATG GAT GAA AGC TGC AGG AAG TCC TCC AAA GCC TGT GGA GCC AGT CTA CCT GAG AAT        255
Ser Gln Trp Val Pro Leu Met Asp Glu Ser Cys Arg Lys Ser Ser Lys Ala Cys Gly Ala Ser Leu Pro Glu Asn         85

TTC TCC ATA AGT GAA ATA TTC AGC CAA GCC ATG GCA GCA AGA AGC ATC AAC CGC CCC ATG CTT CAG GCA GCT GCT        330
Phe Ser Ile Ser Glu Ile Phe Ser Gln Ala Met Ala Ala Arg Ser Ile Asn Arg Pro Met Leu Gln Ala Ala Ala        110

GCT CTC AAA AAG AAA GGA TTC ACA ACG TGC ATT GTC ACC AAC AAC TGG CTG GAC GAC AGT GAC AAG AGA GAC ATC        405
Ala Leu Lys Lys Lys Gly Phe Thr Thr Cys Ile Val Thr Asn Asn Trp Leu Asp Asp Ser Asp Lys Arg Asp Ile        135

CTG GCC CAG ATG ATG TGT GAG CTG AGC CAA CAC TTT GAC TTC CTC ATA GAG TCC TGT CAG GTC GGG ATG ATC AAG        480
Leu Ala Gln Met Met Cys Glu Leu Ser Gln His Phe Asp Phe Leu Ile Glu Ser Cys Gln Val Gly Met Ile Lys        160

CCT GAG CCT CAG ATC TAC AAG TTT GTA CTG GAC ACC CTG AAG GCA AAA CCC AAT GAG GTT GTT TTC CTA GAT GAC        555
Pro Glu Pro Gln Ile Tyr Lys Phe Val Leu Asp Thr Leu Lys Ala Lys Pro Asn Glu Val Val Phe Leu Asp Asp        185

TTT GGA AGT AAT CTG AAG CCA GCC CGT GAC ATG GGG ATG GTT ACC ATC CTG GTC CGC GAC ACA GCC TCG GCT TTG        630
Phe Gly Ser Asn Leu Lys Pro Ala Arg Asp Met Gly Met Val Thr Ile Leu Val Arg Asp Thr Ala Ser Ala Leu        210

AGA GAA CTG GAG AAA GTC ACA GGG ACA CAG TTT CCT GAG GCA CCT CTG CCA GTC CCG TGC AGT CCA AAT GAT GTC        705
Arg Glu Leu Glu Lys Val Thr Gly Thr Gln Phe Pro Glu Ala Pro Leu Pro Val Pro Cys Ser Pro Asn Asp Val        235

AGC CAT GGG TAT GTG ACA GTG AAG CCA GGG ATC CGT CTG CAC TTT GTG GAG ATG GGC TCT GGC CCT GCT ATA TGC        780
Ser His Gly Tyr Val Thr Val Lys Pro Gly Ile Arg Leu His Phe Val Glu Met Gly Ser Gly Pro Ala Ile Cys        260

CTC TGT CAT GGG TTT CCT GAG AGC TGG TTT TCT TGG AGG TAC CAG ATC CCT GCT CTG GCC CAG GCG GGC TTT CGT        855
Leu Cys His Gly Phe Pro Glu Ser Trp Phe Ser Trp Arg Tyr Gln Ile Pro Ala Leu Ala Gln Ala Gly Phe Arg        285

GTT CTA GCT ATA GAC ATG AAA GGC TAT GGA GAC TCA TCT TCT CCT CCA GAA ATA GAA GAA TAT GCT ATG GAA TTG        930
Val Leu Ala Ile Asp Met Lys Gly Tyr Gly Asp Ser Ser Ser Pro Pro Glu Ile Glu Glu Tyr Ala Met Glu Leu        310

CTG TGT GAG GAG ATG GTG ACA TTC CTG AAT AAA CTG GGA ATC CCT CAA GCA GTG TTC ATT GGC CAT GAC TGG GCT       1005
Leu Cys Glu Glu Met Val Thr Phe Leu Asn Lys Leu Gly Ile Pro Gln Ala Val Phe Ile Gly His Asp Trp Ala        335

GGT GTG CTG GTG TGG AAT ATG GCT CTC TTC CAC CCT GAG AGA GTG AGG GCT GTG GCC AGT TTG AAC ACT CCA TTA       1080
Gly Val Leu Val Trp Asn Met Ala Leu Phe His Pro Glu Arg Val Arg Ala Val Ala Ser Leu Asn Thr Pro Leu        360

ATG CCA CCA AAT CCT GAG GTG TCC CCC ATG GAA GTT ATC AGA TCG ATC CCA GTT TTC AAC TAT CAG CTG TAC TTT       1155
Met Pro Pro Asn Pro Glu Val Ser Pro Met Glu Val Ile Arg Ser Ile Pro Val Phe Asn Tyr Gln Leu Tyr Phe        385

CAA GAG CCA GGA GTG GCT GAG GCT GAA CTG GAA AAG AAC ATG AGT CGG ACT TTC AAA AGC TTC TTC CGA ACC AGT       1230
Gln Glu Pro Gly Val Ala Glu Ala Glu Leu Glu Lys Asn Met Ser Arg Thr Phe Lys Ser Phe Phe Arg Thr Ser        410

GAT GAT ATG GGT CTC CTC ACT GTG AAT AAA GCC ACT GAA ATG GGG GGA ATC CTT GTG GGA ACT CCA GAA GAT CCC       1305
Asp Asp Met Gly Leu Leu Thr Val Asn Lys Ala Thr Glu Met Gly Gly Ile Leu Val Gly Thr Pro Glu Asp Pro        435

AAG GTC AGC AAA ATT ACT ACT GAG GAG GAA ATA GAG TAT TAC ATA CAG CAG TTC AAG AAG TCT GGC TTC AGA GGC       1380
Lys Val Ser Lys Ile Thr Thr Glu Glu Glu Ile Glu Tyr Tyr Ile Gln Gln Phe Lys Lys Ser Gly Phe Arg Gly        460

CCT CTA AAC TGG TAT CGA AAC ACA GAA AGA AAC TGG AAG TGG AGC TGT AAG GCG TTG GGA AGG AAG ATC TTG GTC       1455
Pro Leu Asn Trp Tyr Arg Asn Thr Glu Arg Asn Trp Lys Trp Ser Cys Lys Ala Leu Gly Arg Lys Ile Leu Val        485

CCT GCC CTG ATG GTC ACA GCT GAG AAG GAC ATT GTA CTC CGT CCT GAA ATG TCC AAG AAC ATG GAA AAC TGG ATC       1530
Pro Ala Leu Met Val Thr Ala Glu Lys Asp Ile Val Leu Arg Pro Glu Met Ser Lys Asn Met Glu Asn Trp Ile        510

CCT TTC CTG AAA AGG GGA CAC ATC GAA GAC TGT GGT CAC TGG ACA CAG ATA GAG AAA CCG GCA GAG GTG AAC CAG       1605
Pro Phe Leu Lys Arg Gly His Ile Glu Asp Cys Gly His Trp Thr Gln Ile Glu Lys Pro Ala Glu Val Asn Gln        535

ATT CTC ATC AAG TGG CTG AAG ACT GAA ATC CAG AAC CCA TCG GTG ACC TCC AAG ATT TAG CCAGTGGCGTGTCCTCTGC       1684
Ile Leu Ile Lys Trp Leu Lys Thr Glu Ile Gln Asn Pro Ser Val Thr Ser Lys Ile ***                             554

TGGGGACACATTTTCATTTCTGGACGTGGCCTTATCCACAGCCAGCAGCATCGTTCTTTTGCCAGCAGTGATTTTCTTTAAATGAAAATGATCAGATGT       1783
GATGTAATTTTAGATCAGGAAGAAAGTGGTGTGTCTGATTCTTTTGAGGATGACTGTATCACCAAAGGAGAGATCACACCCCAATAGGGAGGCATGGGG       1882
CAGCCCAGTTTGTACCTTTGTAGCCAAACCCAAGCCTGCTCTTTCTGAAGCAGCTGATCAGAGAGTAGGGaccttcattcaataaagctaaggccttgg       1981
tgctcaaaaaaaaaaaaaa                                                                                        2000
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FIG. 2. **Nucleotide sequence and deduced amino acid sequence of rat liver cytosolic epoxide hydrolase.** The numbers to the right of the sequence indicate the position of the last nucleotide/amino acid of the respective row. The numbering starts with position 1 from the beginning of translation for both the nucleotide and amino acid sequences. The leucine zipper motif at the amino terminus and the peroxisomal targeting sequence at the carboxyl terminus are *underlined*. The nucleotide sequence given in *upper-case letters* represents the cDNA sequence of clone pUCcEH1.

cEH protein as potentially being in α-helical conformation, including most of the leucine zipper motif, which, however, contains 2 proline residues. Hydropathy analysis as described by Kyte and Doolittle (44) revealed that cEH appears to be quite hydrophobic, despite its soluble nature. The hydropathy plot of cEH is shown in Fig. 3 in comparison to that of microsomal epoxide hydrolase and glutathione *S*-transferase subunit 3 to illustrate this observation. Among these three proteins, cEH is clearly the one with the most hydrophobic character. The ranking of the average hydropathy values is −0.159 (cEH) > −0.373 (microsomal epoxide hydrolase) > −0.564 (glutathione *S*-transferase).

Northern Blot Analysis—The time course of cEH mRNA induction in rat liver by the potent peroxisome proliferator fenofibrate was monitored by Northern blotting with isolated total RNA. Using the digoxigenin-labeled cDNA insert of pUCcEH1 as the hybridization probe, one single signal per lane was observed (Fig. 4). While the cEH expression in the control rat liver appeared to be below the level of detection under the experimental conditions applied, a significant signal was obtained after 1 day of treatment and apparently reached maximal intensity after 2 days of treatment. From then on, the expression remained constantly enhanced over the whole treatment period, *i.e.* for (at least) 12 days.

Functional Expression in E. coli—Cytosolic epoxide hydrolase was expressed in bacteria using the T7 polymerase system

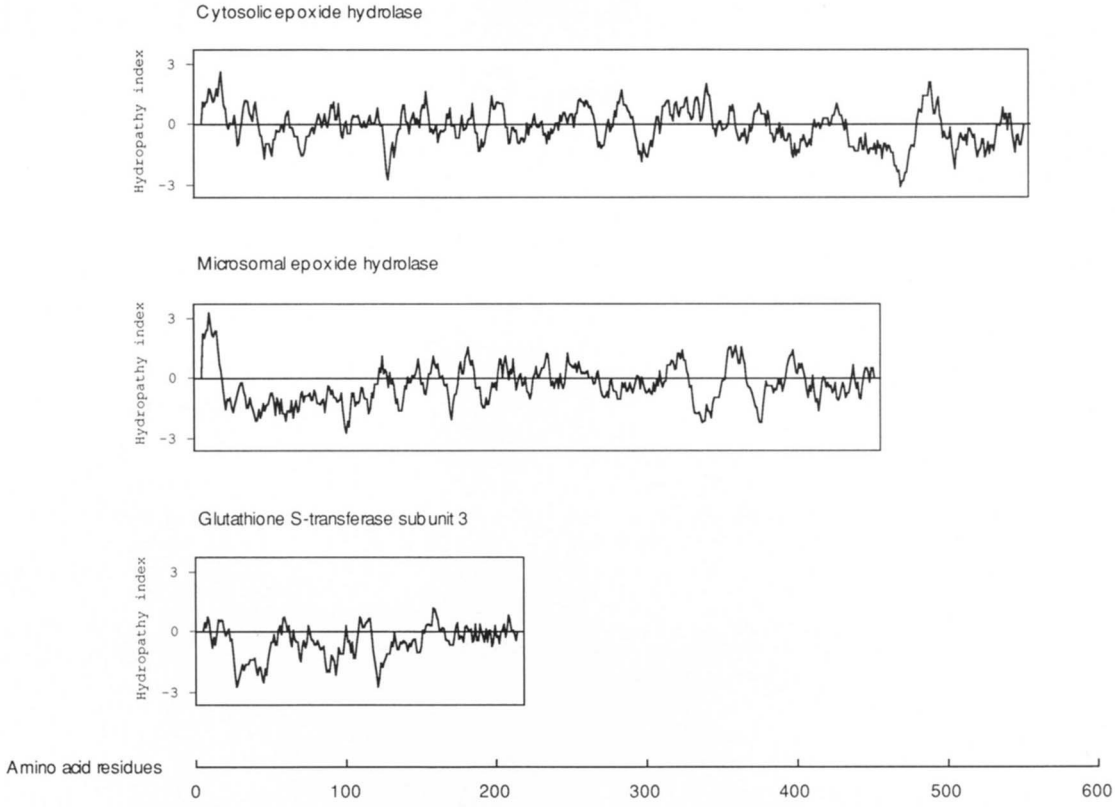

FIG. 3. **Hydropathy profiles of rat liver cytosolic epoxide hydrolase, rat liver microsomal epoxide hydrolase, and rat liver glutathione *S*-transferase subunit 3.** The hydrophobicity profiles were determined according to Kyte and Doolittle (44) using a sliding window of 11 amino acid residues. Positive numbers indicate hydrophobic areas, and negative numbers indicate hydrophilic regions. Of the three proteins compared in this analysis, cEH is clearly the one with the most hydrophobic character (ranking of the average hydropathy values: −0.159 (cEH) > −0.373 (microsomal epoxide hydrolase) > −0.564 (glutathione *S*-transferase)).

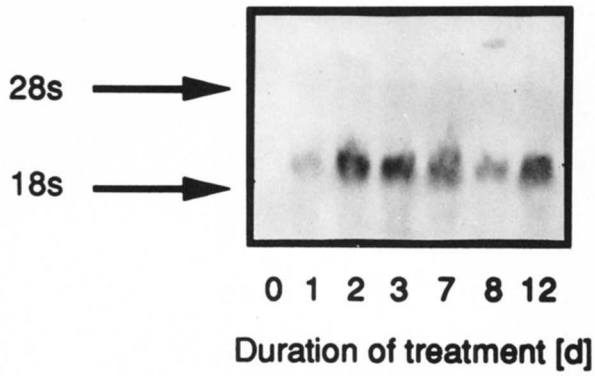

FIG. 4. **Northern blot analysis of time course of cytosolic epoxide hydrolase induction by fenofibrate.** Rat liver total RNA (10 μg/lane) was electrophoresed, blotted, and hybridized with a digoxigenin-labeled cEH cDNA probe as described under "Experimental Procedures." The number below each lane gives the duration of the fenofibrate treatment of the respective rat in days. The *arrows* mark the positions of the ribosomal RNAs.

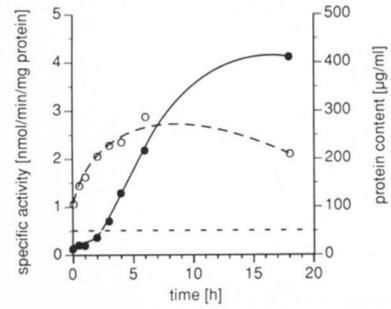

FIG. 5. **Functional expression of cytosolic epoxide hydrolase in bacteria.** Cytosolic epoxide hydrolase was expressed in *E. coli*. Total protein content (○) and specific enzymatic activity of cytosolic epoxide hydrolase (●) were determined over a period of 18 h after initiation of cEH expression. The *dashed line* represents the average enzymatic activity in liver cytosols of maximally induced Sprague-Dawley rats. Under identical experimental conditions, no enzymatic activity was detectable in bacteria harboring the expression vector lacking the cEH cDNA insert. For experimental details, see "Experimental Procedures."

(45). Under permissive conditions, cEH protein accumulated in enzymatically active form over a prolonged period of time as shown in Fig. 5. The crude bacterial suspension reached a specific enzymatic activity of 4 nmol of *trans*-stilbene oxide hydrolyzed per min/mg of protein at 18 h post-induction, which exceeds the value obtained with a maximally induced rat liver cytosol by a factor of 8.

DISCUSSION

In this report, the isolation and characterization of a cDNA encoding the entire rat liver cytosolic epoxide hydrolase protein is described. Physicochemical characteristics calculated from the deduced amino acid sequence were in good agreement with data obtained experimentally for the protein: the calculated M_r of 62,268 and pI of 5.8 compare very well to the experimental findings of M_r = 61,000 (by SDS-polyacrylamide gel electrophoresis) and pI 5.5 (by chromatofocusing) (10), and the secondary structure analysis predicting ~40% of the protein being in α-helical conformation is very similar to the estimated helical fraction of 38% for mouse cEH on the basis of CD spectra (30). Furthermore, the inducibility of cEH activity by peroxisome proliferators as observed by several groups in the loose sense of the term as a time-dependent increase in enzyme activity (17, 24, 37) was proven to be related to an increase in the amount of cEH mRNA as demonstrated by Northern blotting.

As reported previously (27), the cEH carries a carboxyl-terminal peroxisomal targeting signal that is functionally impaired due to the exchange of a leucine residue in the original motif with an isoleucine in the cEH sequence. While this modified sequence is supposed to be inactive in mediating the translocation of proteins into peroxisomes according to the results of Gould *et al.* (28), our observations imply that it still functions as an import signal, yet much less efficiently. In our opinion, a residual targeting function of a much less active peroxisomal targeting signal may have escaped detection in the system used by Gould *et al.* A protein carrying such an impaired peroxisomal targeting signal should be present in both cellular compartments, *i.e.* peroxisomes as well as cytosol, in significant amounts, as observed in the case of cEH. Several researchers have speculated about the existence of more than one cEH form, being closely related to the classical enzyme, as a possible explanation for the exceptional bicompartmental location of cEH. While there is yet no significant experimental evidence for a multiplicity of cEH, all data obtained up to now appear to be compatible with the existence of one single cEH protein in view of the modified peroxisomal targeting signal that has been identified at the cEH carboxyl terminus.

Under native conditions, cEH exists in the form of stable homodimers. PROSITE motif analysis of the deduced amino acid sequence has identified a potential leucine zipper, a structural component known to be involved in the dimerization of a number of proteins (42). However, an essential prerequisite for the effectiveness of a leucine zipper motif is the formation of an α-helix of the respective protein domain since only under these conditions do the leucine residues, appearing with a periodicity of 7 in the motif, align properly to form the zipper structure (42). Since 2 proline residues are present in the observed leucine zipper-like sequence, no continuous helix may be expected to be formed as proline usually leads to kinks in the regular secondary structure due to its unique sterical restrictions. Thus, it is unclear whether the identified structure has the potential to be the driving force for the observed dimerization of cEH.

The functional expression of cEH using the T7 RNA polymerase system has resulted in the recovery of high enzymatic activity. On the basis of the specific activity obtained, one may estimate the relative amount of cEH protein to be at least ~1–2% of the total bacterial protein. This expression system therefore has the potential to be used for further studies on modified cEH derivatives to elucidate, for instance, the role of the potential leucine zipper domain in protein dimerization or the importance of other structural components in the catalytic process.

Acknowledgments—We wish to express special thanks to David Grant and Bruce Hammock for communicating results on mouse liver cEH cDNA prior to publication. The expert technical assistance of Heike Wagner is gratefully acknowledged.

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